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☐ 1: J Mol Biol. 1999 May 21;288(5):825-36.

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## Alterations in the directionality of lambda site-specific recombination catalyzed by mutant integrases in vivo.

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Phage lambda integrative and excisive recombination normally proceeds by a pair of sequential strand exchanges. During the first exchange reaction, the "top" strand in each recombination site is cleaved, exchanged, and religated generating a Holliday junction intermediate. This intermediate DNA structure is resolved through a pair of reciprocal "bottom" strand exchanges, leading to recombinant products. The strict co-ordination of exchange reactions ensures religation between correct partner strands only. Here we show that the directionality of recombination is altered in vivo by two mutant integrases, Int-h (E174 K) and a double mutant Int-h/218 (E174 K/E218 K). This change in directionality leads to deletion instead of inversion on substrates that carry inverted attachment sites and, depending on the pair of target sites employed, requires the presence or absence of integration host factor. Neither Fis nor Xis is involved in deletion. Sequence analyses of deletion products reveal that the newly generated hybrid attachment site exhibits a reversed genetic polarity. We demonstrate that only one of two possible hybrid site configurations is generated and discuss two pathways leading to deletion. In the first, deletion results from a wrong alignment of the two recombination sites within the synaptic complex. In the second pathway, the unco-ordinated cleavage by the mutant integrases of all four DNA strands present in a conventional Holliday junction intermediate leads to two double-stranded breaks, whereby the subsequent rejoining between "wrong" partner strands appears restricted to only two strands. Copyright 1999 Academic Press.

PMID: 10329182 [PubMed - indexed for MEDLINE]